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Entry of ricin and Shiga toxin into cells: molecular mechanisms and medical perspectives

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contents b

Abstract

A large number of plant and bacterial toxins with enzymatic activity on intracellular targets are now known. These toxins enter cells by first binding to cell surface receptors, then they are endocytosed and finally they become translocated into the cytosol from an intracellular compartment. In the case of the plant toxin ricin and the bacterial toxin Shiga toxin, this happens after retrograde transport through the Golgi apparatus and to the endoplasmic reticulum. The toxins are powerful tools to reveal new pathways in intracellular transport. Furthermore, knowledge about their action on cells can be used to combat infectious diseases where such toxins are involved, and a whole new field of research takes advantage of their ability to enter the cytosol for therapeutic purposes in connection with a variety of diseases. This review deals with the mechanisms of entry of ricin and Shiga toxin, and the attempts to use such toxins in medicine are discussed.

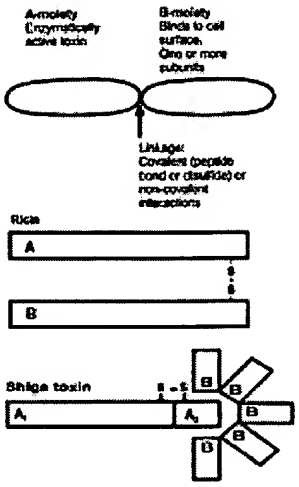
Keywords: endocytosis, endoplasmic reticulum, Golgi apparatus, ricin, Shiga toxin

Introduction

The plant toxin ricin and the bacterial Shiga toxin are members of a family of protein toxins that are highly toxic to a large

number of eukaryotic cells (Sandvig and van Deurs, 1996, 1999; Acheson and Keusch, 1999; Olsnes *et al.*, 1999). Although such toxins are found in different plants and bacteria, they have several common characteristics. They have one moiety responsible for binding to the cell surface, whereas another moiety enters the cytosol and inactivates protein synthesis enzymatically (Figure 1). The crystallographic structures of ricin (Rutenber *et al.*, 1991) and Shiga toxin (Fraser *et al.*, 1994) with their respective binding (B) and enzymatically active (A) moieties are shown in Figure 2. The cytosolic target of ricin and Shiga toxin is the 28S RNA of the 60S ribosomal subunit (Endo *et al.*, 1987). Reduction of the disulfide bond connecting the A- and B-moieties of ricin (yellow area in Figure 2A) is required for optimal enzymatic activity (Sandvig and van Deurs, 1996). In the case of Shiga toxin, proteolytic cleavage of the A-moiety in a loop formed by the internal disulfide bond (yellow area in Figure 2B) and reduction of this disulfide bond facilitate rapid intoxication (Sandvig and van Deurs, 1996). In most cells, the processing of the Shiga toxin A-moiety is performed by the enzyme furin (Garred *et al.*, 1995), located in the Golgi apparatus and in endosomes. This enzyme recognizes an amino acid sequence found not only in the loop of the Shiga toxin A-moiety, but also in a number of other bacterial toxins where proteolytic cleavage is important for activation of the toxin (Gordon and Leppla, 1994). Similar two-moiety structures to those of ricin and Shiga toxin are found in bacterial toxins such as diphtheria toxin and *Pseudomonas* exotoxin A (Wick *et al.*, 1990; Sandvig and Olsnes, 1991; Olsnes *et al.*, 1999; Pizza *et al.*, 1999). In all cases, these toxins become endocytosed after binding to the cell surface. After transport to different intracellular destinations, they cross the membrane and exert their toxic effect in the cytosol. Whereas ricin and Shiga toxin attack ribosomes, diphtheria toxin and *Pseudomonas* exotoxin A inactivate elongation factor 2 (EF2) and thereby inhibit protein synthesis. Both ricin and Shiga toxin are transported in a retrograde manner to the endoplasmic reticulum (ER) before being translocated to the cytosol (Sandvig and van Deurs, 1996, 1999; Rapak *et al.*, 1997; Arab and Lingwood, 1998; Lingwood *et al.*, 1998; Girod *et al.*, 1999; White *et al.*, 1999). Ricin and the other members of this toxin family are very efficient at cell killing. For instance, one molecule of ricin can inactivate ~2000 ribosomes/min. Since the toxins are also quite stable, one or a few molecules in the cytosol are sufficient to kill a cell (Olsnes and Sandvig, 1988). In addition to having a direct effect on protein synthesis, protein toxins can also induce DNA cleavage and cause apoptosis-like changes in cells (Sandvig and van Deurs, 1996), and new studies indicate that in the case of Shiga toxin this process may be regulated by proteins of the Bcl-2 family (Jones *et al.*, 2000; Suzuki *et al.*, 2000).

Figure 1
Schematic structure of protein toxins. The top drawing applies to a number of different toxins



such as the bacterial toxins *Pseudomonas* exotoxin A (Wick *et al.*, 1990; Pizza *et al.*, 1999), diphtheria toxin (Sandvig and Olsnes, 1991; Olsnes *et al.*, 1999; Pizza *et al.*, 1999), cholera toxin (Holmgren, 1981; Fishman and Orlandi, 1994; Pizza *et al.*, 1999) and Shiga toxin (Sandvig and van Deurs, 1996, 1999; Acheson and Keusch, 1999), as well as the plant toxins ricin (Sandvig and van Deurs, 1996, 1999; Olsnes *et al.*, 1999), abrin, modeccin, volkensin and viscumin (Sandvig and van Deurs, 1996, 1999; Olsnes *et al.*, 1999). In addition, ricin with its disulfide bond between the A- and B-moieties, and Shiga toxin with five subunits in the B-moiety are shown.

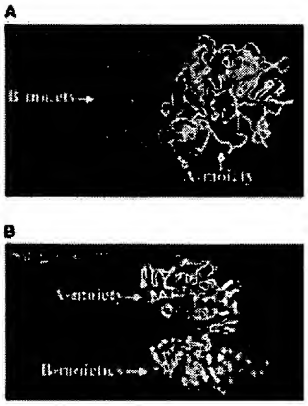


Figure 2
Crystallographic structures of ricin (**A**) and Shiga toxin (**B**). The enzymatically active subunits are in green, whereas the binding moiety of ricin is in red, and the five small binding subunits of Shiga toxin are multicoloured. The disulfide bond (and the neighbouring carbon atoms) connecting the two chains of ricin, and the internal disulfide bond in the A-moiety of Shiga toxin are yellow. The structures have been obtained from the PDB protein data bank (ricin: 1DMO; Shiga toxin:2AA1), and are based on work published by Rutenber *et al.* (1991) and Fraser *et al.* (1994).

The protein toxins have proven valuable in studies of endocytic processes and intracellular transport in general. As described below, ricin has been used to study different types of endocytosis (Llorente *et al.*, 1998a; Rodal *et al.*, 1999; Sandvig and van Deurs, 1999), and the toxin has been useful in studies of membrane transport to various intracellular destinations such as the Golgi apparatus and the ER, as well as to late endosomes/lysosomes and across polarized epithelial cell layers (Sandvig and van Deurs, 1996, 1999; Rapak *et al.*, 1997; Llorente *et al.*, 1998a, b). Investigations of Shiga toxin have revealed novel apects of glycolipid transport. Results obtained with this toxin show that it is not only protein sequences that are of importance for intracellular sorting, but also the glycosphingolipid composition (Sandvig *et al.*, 1994; Sandvig and van Deurs, 1996; Arab and Lingwood, 1998). In fact, studies of Shiga toxin were the first to reveal that a molecule can be transported from the cell surface through the Golgi apparatus and to the ER (Sandvig *et al.*, 1992). The toxins can also be used to study translocation to the cytosol, since the activity of even a few toxin molecules can be monitored as an inhibition of protein synthesis. Thus, ricin and Shiga toxin can be used to investigate transport from the ER, and other toxins, such as diphtheria toxin, can be used to

study transport from endosomes or across the plasma membrane (Sandvig and Olsnes, 1991; Olsnes *et al.*, 1999; Pizza *et al.*, 1999).

A number of infectious diseases are associated with secretion of toxins (e.g. diphtheria and dysentery), and the extreme toxicity of the toxin molecules contributes greatly to the severity of such diseases. Although in many cases infectious diseases are under reasonable control today, Shiga toxin, which is secreted by *Shigella dysenteriae*, and the almost identical Shiga-like toxin 1 and related Shiga-like toxin 2 secreted by *Escherichia coli* and other bacteria are responsible for widespread disease and for the deaths of a large number of people on a worldwide basis (Takeda *et al.*, 1993; Kaper, 1998; Bower, 1999; Uchida *et al.*, 1999; Kitov *et al.*, 2000; Paton *et al.*, 2000). Shiga-like toxins have received considerable attention during the last decade. They have become an increasing threat to human health also in developed countries where they are responsible for the so-called 'hamburger' disease. Bacteria secreting Shiga-like toxins can contaminate different types of food, including milk, apple juice and vegetables (Kaper, 1998; Bower, 1999; Uchida *et al.*, 1999). Infections with these bacteria may lead to haemolytic uraemic syndrome and kidney failure, particularly in children (Bower, 1999; Uchida *et al.*, 1999). Clarification of the mechanism of action of Shiga toxin and other bacterial toxins in different cell types is therefore warranted in order to control the diseases (Kitov *et al.*, 2000; Paton *et al.*, 2000). Knowledge of toxin–receptor interactions at the molecular level provides us with tools to treat such infectious diseases. Recent publications report the development of bacteria with a Shiga toxin receptor mimic that binds and thereby neutralizes Shiga toxin (Paton *et al.*, 2000), and the production of a pentamer of trisaccharides that efficiently binds Shiga toxin (Kitov *et al.*, 2000).

Medical research is now also taking advantage of the unique properties of ricin and Shiga toxin as well as other protein toxins in order to develop novel therapeutics for other diseases. Ricin and other toxins that intoxicate different cell types rather non-specifically can be targeted to specific cells by coupling the enzymatically active part of the molecule to other ligands or to antibodies directed against, for instance, cancer cells (Frankel *et al.*, 1996; Laske *et al.*, 1997; Kreitman, 1999; Kitov *et al.*, 2000; Paton *et al.*, 2000; and see below). Furthermore, as discussed herein, the toxins (or parts of the toxins) can be used as vectors to bring other molecules into cells, an ability of toxins that is of great importance in cell biology as well as medicine.

End cytosis f ricin and Shiga t xin

Since the plant toxin ricin binds to both glycolipids and glycoproteins with terminal galactose all over the cell surface and is therefore localized to all types of membrane

invaginations (Sandvig and van Deurs, 1996), the toxin is presumably internalized by all endocytic mechanisms operating in a given cell (Sandvig and van Deurs, 1996, 1999) (Figure 3). Ricin has been localized in clathrin-coated pits, but is still endocytosed when this pathway is blocked (Sandvig and van Deurs, 1996). It was actually by using ricin that some of the early evidence for a clathrin-independent endocytic mechanism was obtained (Moya *et al.*, 1985; Sandvig *et al.*, 1987).

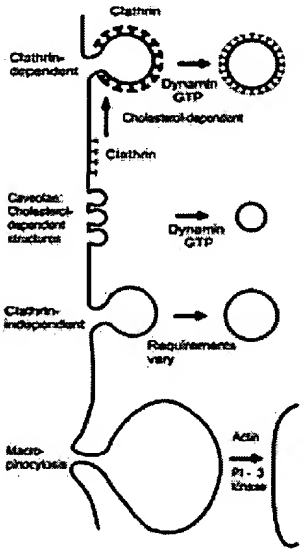


Figure 3
Structures proposed to be involved in endocytosis.

It is now clear that clathrin-independent endocytosis can also be different from uptake by caveolae and macropinocytosis (Sandvig and van Deurs, 1999). For instance, clathrin-independent endocytosis occurs on the apical side of polarized MDCK cells (Eker *et al.*, 1994; Holm *et al.*, 1995; Llorente *et al.*, 1996, 2000), whereas all of the morphologically identifiable, deeply invaginated caveolae in this cell type are localized in the basolateral domain (Vogel *et al.*, 1998). Furthermore, clathrin-independent endocytosis of ricin occurs even when uptake from caveolae and clathrin-dependent endocytosis are inhibited by extraction of membrane cholesterol (Rodal *et al.*, 1999). Removal of cholesterol leads to the disappearance of caveolae (Rothberg *et al.*, 1990) and inhibits formation of invaginated clathrin-coated pits (Rodal *et al.*, 1999; Subtil *et al.*, 1999).

Clathrin-independent endocytosis seems to comprise more than one mechanism (Herskovits *et al.*, 1993; van der Bliek *et al.*, 1993; Artalejo *et al.*, 1995; Damke *et al.*, 1995; Sandvig and van Deurs, 1996; Llorente *et al.*, 2000), and clearly clathrin-independent endocytosis of ricin in a polarized cell can be differentially regulated on the apical and basolateral poles (Sandvig and van Deurs, 1996; Llorente *et al.*, 2000). So far, it is not known whether the endocytic machinery is the same but subjected to different regulation. The molecular mechanisms behind clathrin-independent endocytosis have not yet been clarified, but this type of endocytosis is involved in the uptake

of physiological molecules as well as infectious agents. Similarly to ricin, Moloney murine leukaemia virus (Lee and Anderson, 1999), angiotensin (Zhang *et al.*, 1996), muscarinic receptors (Pals-Rylaarsdam *et al.*, 1997) and D2 dopamine receptors (Vickery and Zastrow, 1999) are endocytosed upon expression of mutant dynamin, which blocks entry both from clathrin-coated pits (Herskovits *et al.*, 1993; van der Bliek *et al.*, 1993; Damke *et al.*, 1995) and from caveolae (Henley *et al.*, 1998; Oh *et al.*, 1998).

In contrast to ricin, Shiga toxin is endocytosed preferentially by the clathrin-coated pathway, although it is bound to a glycolipid receptor (globotriasylceramide; Gb3) (Sandvig and van Deurs, 1996). The aggregation of the toxin–Gb3 complex in these pits is mediated by the toxin by a so far unknown mechanism (Sandvig and van Deurs, 1996).

A commonly asked question is: how much do the different endocytic pathways contribute to membrane uptake? Studies of toxin uptake may help to answer this question. Although the area of the plasma membrane occupied by clathrin-coated pits and caveolae differs greatly from one cell type to another (van Deurs *et al.*, 1989), and interfering with one type of endocytosis might actually up-regulate another mechanism (Damke *et al.*, 1995; Llorente *et al.*, 1998a), ricin endocytosis is reduced to ~50% under several sets of conditions that interfere with the clathrin-dependent pathway (Sandvig and van Deurs, 1996, 1999). This suggests that ~50% of membrane uptake may also occur by a clathrin-independent endocytosis under normal conditions.

Transport of toxins to the Golgi apparatus

As described above (and shown in Figure 4), ricin and Shiga toxin have to pass through the Golgi apparatus on their way to the cytosol. A relatively small fraction of the endocytosed toxin molecules are transported to the Golgi apparatus (Sandvig and van Deurs, 1996; van Deurs *et al.*, 1988) (Figure 4). However, Golgi modification (sulfation), cell fractionation or microscopy can be used to monitor this transport (Sandvig and van Deurs, 1996; Rapak *et al.*, 1997; Llorente *et al.*, 1998a,b). Toxins were actually the first molecules demonstrated to go from endosomes to the Golgi apparatus (Gonatas *et al.*, 1980; Sandvig and van Deurs, 1996). Importantly, such studies reveal that it is not only transport from the Golgi apparatus to the plasma membrane that is under regulation (Pimplikar and Simons, 1993; Keller and Simons, 1997). Transport from endosomes to the Golgi apparatus is also regulated, and it can even be differentially regulated depending on whether the toxins are entering from the apical or the basolateral pole of a cell (Sandvig and van Deurs, 1996; Llorente *et al.*, 1998b), implying that they may reach the Golgi complex from different endocytic compartments. Interestingly, transport of ricin and Shiga toxin to the Golgi apparatus is not dependent on low endosomal pH (Melby *et al.*, 1991; Sandvig and van Deurs,

1996; Schapiro *et al.*, 1998), indicating that this transport step occurs independently of the low pH-dependent formation of carrier vesicles reported to operate between early and late endosomes in some cells (Clague *et al.*, 1994).

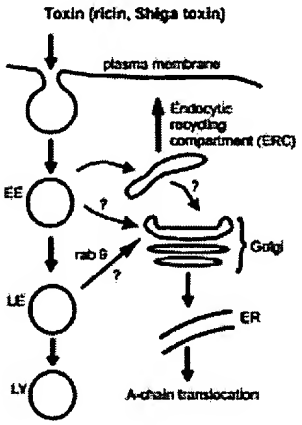


Figure 4
Some intracellular transport routes followed by protein toxins. Question marks indicate routes for toxin transport currently under debate (see text). EE, early endosomes; LE, late endosomes; LY, lysosomes; ER, endoplasmic reticulum.

What is the route that ricin and Shiga toxin use on their way to the Golgi apparatus? The routing of these two toxins to the Golgi apparatus could actually differ. Ricin could follow membrane bulk transport and thereby use more than one pathway, whereas aggregation of Shiga toxin in specialized membrane domains might be required not only for uptake from the cell surface (Sandvig and van Deurs, 1996), but also for transport from endosomes. It was suggested recently that Shiga toxin goes to the Golgi apparatus via the perinuclear endocytic recycling compartment (ERC), and that the transport of Shiga toxin from the ERC to the Golgi apparatus occurs via clathrin-coated structures containing AP1 (Mallard *et al.*, 1998) (Figure 4).

It might be difficult to follow toxin transport using only microscopy since failure to visualize a certain ligand in a given compartment could be due to rapid transport out of this compartment. Thus, to exclude trafficking in a certain direction, one may have to interfere with the transport machinery responsible for trafficking at this location. Along these lines, we have recently generated HeLa cells with inducible expression of a dominant-negative mutant of Rab9 (Goda and Pfeffer, 1988; Lombardi *et al.*, 1993) that inhibits late endosome-to-Golgi transport of the mannose-6-phosphate receptor. Interestingly, ricin transport to the Golgi apparatus, measured as sulfation of modified ricin, was not inhibited by mutant Rab9 (T.-G.Iversen, A.Llorente, P.Nicoziani, B.van Deurs and K.Sandvig, in preparation), implying that ricin can enter the Golgi apparatus by mechanisms different from that of the mannose-6-phosphate receptor. Much work remains before toxin transport to the Golgi apparatus is completely understood, but it is most likely that such studies will provide us with information relevant for transport of molecules that are physiologically important.

Even though the routing of toxins to the Golgi apparatus is not clarified, the toxins can be used to investigate the Golgi apparatus itself. For example, fluorescent derivatives of the non-toxic B-fragment of Shiga toxin were used recently to study the pH of both the Golgi apparatus and the ER (Kim *et al.*, 1998; Schapiro and Grinstein, 2000).

Retrograde transport of toxin through the Golgi apparatus and translocation to the cytosol

A pathway leading all the way from the cell surface to the Golgi apparatus and the ER was first detected by studying Shiga toxin transport (Sandvig *et al.*, 1992). Later on, similar transport was also shown for ricin (Rapak *et al.*, 1997) and cholera toxin (Majoul *et al.*, 1996; Sandvig *et al.*, 1996). How are toxins transported retrogradely through the Golgi apparatus and to the ER? Although the mechanisms of transport within the Golgi apparatus and retrieval of membrane proteins containing the sequence KDEL from Golgi to the ER via COPI-coated vesicles have been studied extensively (Warren and Malhotra, 1998; Pelham, 2000), a number of basic questions are still not answered. For instance, how many mechanisms and pathways can be used by molecules going from the *trans*-Golgi network (TGN) to the ER? Investigations of toxins have revealed that more than one mechanism exists (Jackson *et al.*, 1999; Johannes and Goud, 2000), and the various toxins seem to use different mechanisms.

Both cholera toxin (Lencer *et al.*, 1995; Majoul *et al.*, 1996) and *Pseudomonas* exotoxin A (Seetharam *et al.*, 1991; Kreitman and Pastan, 1995; Jackson *et al.*, 1999) have a KDEL sequence that can facilitate retrograde transport in general (Lewis and Pelham, 1992; Tang *et al.*, 1992), and that seems to be important for efficient retrograde transport of these toxins, whereas neither ricin (Lamb *et al.*, 1985) nor Shiga toxin (Seidah *et al.*, 1986; Kozlov *et al.*, 1987; Strockbine *et al.*, 1988) has such a sequence. It was recently determined that expression of lysozyme–KDEL, which leads to a redistribution of the KDEL receptor from the Golgi complex to the ER, interferes with the intoxication with *Pseudomonas* exotoxin A, but has no effect on the toxicity of Shiga-like toxin-1 (Jackson *et al.*, 1999), which is almost identical to Shiga toxin (Takeda *et al.*, 1993). In fact, a Rab6-dependent, COPI-independent retrograde transport route used by the Shiga B-chain has been described recently (Girod *et al.*, 1999; White *et al.*, 1999). Studies of toxin transport might thus be useful to clarify the unresolved question of retrograde transport within the Golgi complex.

Since the enzymatically active part of the toxins is the A-moiety (Figures 1 and 2), it is clear that this part has to reach the cytosol. Until recently, it was assumed that the B-moiety remained in the ER. Interestingly, it was reported that Shiga B-chain has the ability to induce DNA cleavage and apoptosis in fibroblasts upon regulated expression in these cells, whereas

the A-chain is unable to induce degradation of DNA, suggesting that Shiga B-chain translocation to the cytosol might occur and produce additional effects (Nakagawa *et al.*, 1999).

What is the mechanism involved in toxin translocation from the ER to the cytosol? Studies of cells with mutations in the transporter associated with antigen processing (TAP) suggested that this system is not involved in toxin translocation (Sandvig and van Deurs, 1996). During recent years, the ability of the heterotrimeric protein complex Sec61p (known to be required for protein translocation from the cytosol to the ER; Matlack *et al.*, 1998) to translocate proteins from the ER to the cytosol, even after glycosylation, has been well characterized (Suzuki *et al.*, 1998; Cacan and Verbert, 1999). This protein complex might be involved in toxin translocation. In fact, ricin A-chain seems to interact with this complex since it can be co-immunoprecipitated with Sec61α (Wesche *et al.*, 1999). Furthermore, a ricin A-chain mutant that was translocated into the yeast ER after synthesis in the same cell seemed to re-enter the cytosol by the Sec61p translocon (Simpson *et al.*, 1999). In addition to being an ER component, Sec61 has recently been found in the ERGIC (ER–Golgi intermediary compartment), and the possibility exists that toxins might be translocated from this compartment, for instance after reduction/interaction with ER proteins such as disulfide isomerase/chaperones. It has actually been suggested that misfolded major histocompatibility complex (MHC) class I proteins might be transported to the cytosol from this destination (Greenfield and High, 1999). The toxins described here clearly exploit the cell machinery to gain access to their cytosolic target.

So far, little is known about the molecular details of toxin transport across the membrane, but unfolding of the A-moiety and exposure of a hydrophobic stretch might be required for transport to the cytosol (Lord and Roberts, 1998). Mutations in this area inhibit translocation without reducing the catalytic activity of the toxin (Lord and Roberts, 1998).

As illustrated above, it is amazing what studies of toxins can reveal about transport processes in cells. An important question is to what extent these pathways are used by other types of molecules. For instance, do proteins other than toxins take advantage of retrograde transport from the cell surface to the ER? The answer seems to be yes. Recent studies revealed retrograde transport of CD19, a molecule with sequence similarity to the Shiga B-chain (Khine *et al.*, 1998). CD19 actually binds to Gb3, the Shiga toxin receptor, and retrograde transport of CD19 seems to be important for induction of apoptosis in B cells (Khine *et al.*, 1998). Furthermore, a number of growth factors are transported to the nucleus (Pederson, 1998). However, it is still not known whether retrograde transport through the Golgi apparatus is involved in such transport.

Toxins as t ls in medicine

It is an old idea that protein toxins might be targeted to certain cell types such as cancer cells and serve as 'magic bullets' (Figure 5A). This was proposed originally by Paul Ehrlich (1854–1915) (Ehrlich, 1957). Preparation of toxin conjugates has been facilitated by new technologies and the production of monoclonal antibodies (Frankel *et al.*, 1996; Laske *et al.*, 1997; Kreitman, 1999). Today, a large number of laboratories are working with toxin conjugates, and immunotoxins and toxins conjugated to other ligands are being tested clinically with promising results on both solid tumours and haematological malignancies (Frankel *et al.*, 1996, 2000a, b; Laske *et al.*, 1997; Kreitman, 1999). Interestingly, compounds known to sensitize cells to some of the toxins may also potentiate the action of immunotoxins (Sandvig and van Deurs, 1996). An example is found in a recent article by van Horssen *et al.* (1999), who report that the action of a recombinant ricin A-chain conjugate is potentiated by chloroquine, and propose that this could also be applied *in vivo*. In the case of Shiga toxin, which binds to the glycosphingolipid Gb3, it has been suggested that the toxin might be used even in its native form, since some tumour cells have an increased number of binding sites for the toxin (Lingwood *et al.*, 1998; LaCasse *et al.*, 1999).

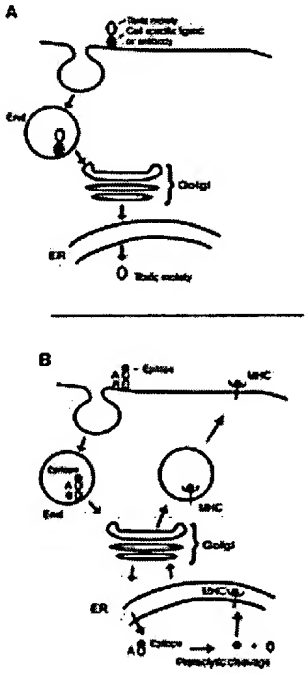


Figure 5
Model of modified toxin molecules entering cells. (**A**) Cell-specific targeting of toxin with translocation from the ER. Binding occurs either via antibodies directed against a certain cell type, or by using ligands that bind to a given cell. (**B**) Toxins transported to the Golgi/ER as vehicles to mediate MHC class I presentation of peptides. End, endosome; A, A-moiety (modified) of toxin (see Figure 1); B, B-moiety of toxin (see Figure 1).

The ability of protein toxins to enter the cytosol can also be exploited to bring other proteins or peptides into the cytosol. Toxin vehicles can be useful for both experimental and therapeutic purposes, and since cells differ in their number of receptors for the various protein toxins (Sandvig and van Deurs, 1996; LaCasse *et al.*, 1999), the availability of more

than one type of translocation system may be necessary to obtain transport into a given cell type. Furthermore, the efficiency of membrane translocation for a protein or peptide is likely to vary depending on the toxin used, since the mechanisms of transport for the various toxins differ. These systems might be used to deliver molecules of a wide range, both larger proteins with enzymatic activity on their own and peptides that can be used to create immunity against virus and tumour cells. Normally, peptides released in the cytosol by proteasomal degradation are transported into the ER by the specialized transporter TAP, are bound by MHC class I receptors and are transported to the cell surface where the complex is recognized by cytotoxic T cells (Williams *et al.*, 1996). The idea is to use toxins to get a peptide into the cytosol, where it would be released and subsequently presented by MHC class I molecules (Figure 5B). There are now several examples of this idea actually working. In one case, *E.coli* heat-labile toxin was demonstrated to deliver both antiviral and antigenic peptides (Marcello *et al.*, 1994; Loregian *et al.*, 1999). Also, a modified form of *Pseudomonas* exotoxin A was used to deliver epitopes to class I MHC molecules (Donnelly *et al.*, 1993). Anthrax toxin-carrying epitopes can induce protective antiviral immunity (Doling *et al.*, 1999), and Shiga-like toxin I (Noakes *et al.*, 1999) can deliver antigenic peptides into cells. A peptide derived from influenza virus matrix protein was added at the DNA level to both the N- and C-terminal ends of a catalytically inactive A-fragment of Shiga toxin. The modified toxin was able to sensitize cells to lysis by cytotoxic T lymphocytes in both cases (Noakes *et al.*, 1999). This might involve translocation of the epitope into the cytosol and subsequent delivery into the ER where binding to MHC class I molecules could take place (Noakes *et al.*, 1999). Even peptides coupled to Shiga toxin B-fragment (Lee *et al.*, 1998) can be presented by MHC class I molecules, but it is not yet known whether epitopes coupled to the B-subunit of the toxin might be released from the B-chain within the ER, or if release occurs after translocation of the B-chain to the cytosol. Altogether, experiments with toxins as tools in creating an immune response are promising.

Concluding comments

Although protein toxins may originally have been regarded only as disease-causing agents, the technology available today provides us with tools to use these toxins to fight disease. They can be used as constituents of immunotoxins or other targeted (cell-specific) chimeric molecules. Furthermore, they can be used as vehicles to bring proteins and peptides into cells, with the possibility of creating immune responses to virus or cancer cells. Clearly, further studies are required in order to clarify the potential of these agents in medicine. Importantly, basic information about transport pathways in cells can be obtained from studies of toxins, and investigations of the roles of the various domains of the protein toxins, as well as increased knowledge about their translocation to the cytosol,

will increase their importance as general tools in cell biology.

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